Acclimation to future atmospheric CO_2 levels increases photochemical efficiency and mitigates photochemistry inhibition by warm temperatures in wheat under field chambers

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Abstract

A study was conducted over two years to determine whether growth under elevated CO_2 (700 μmol mol^{-1}) and temperature (ambient + 4ºC) conditions modifies photochemical efficiency or only the use of electron transport products in spring wheat grown in field chambers. Elevated atmospheric CO_2 concentrations increased crop dry matter at maturity by 12%-17%, while above-ambient temperatures did not significantly affect dry matter yield. In measurements with ambient CO_2 at ear emergence and after anthesis, growth at elevated CO_2 concentrations decreased flag leaf light-saturated carbon assimilation. The quantum yield of electron transport (Φ_{PSII}) measured at ambient CO_2 and higher irradiances increased at ear emergence and decreased after anthesis in plants grown at elevated CO_2. At higher light intensities, but not in low light, photochemical quenching (qP) decreased after growth in elevated CO_2 conditions. Growth under CO_2 enrichment increased dark- (Fv:Fm) and light-adapted (Fv':Fm') photochemical efficiencies, and decreased the chlorophyll a:b ratio, suggesting an increase in light-harvesting complexes relative to PSII reaction centres. A relatively higher decrease in carbon assimilation than the decrease in Φ_{PSII} pointed to a sink other than CO_2 assimilation for electron-transport products at defined growth stages. With higher light intensities, warmer temperatures increased Φ_{PSII} and Fv':Fm' at ear emergence and decreased Φ_{PSII} after anthesis; in ambient -but not elevated- CO_2, warmer temperatures also decreased qP after anthesis. CO_2 fixation increased or did not change with temperature, depending on the growth stage and year.

We conclude that elevated CO_2 decreases the carbon assimilation capacity, but increases photochemistry and resource allocation to light harvesting, and that elevated levels of CO_2 can mitigate photochemistry inhibition due to warm temperatures.

Keywords: acclimation; chlorophyll a:b ratio; chlorophyll fluorescence; carbon assimilation; elevated CO_2; elevated temperature; crop yield; wheat

Introduction

A rise in CO_2 concentration above current atmospheric levels will increase photosynthesis in C_3 plants because Rubisco is not substrate-saturated and oxygenation is inhibited (Long *et al.* 2004). Under elevated CO_2, photosynthetic electron transport destined for CO_2 assimilation will increase proportionately, while electron flux to oxygen will be inhibited. The net result of these changes will determine whether total electron flux increases (Hymus *et al.* 2001) if there is no effect of elevated CO_2 on alternative electron sinks (Habash *et al.* 1995; Hymus *et al.* 1999). An acclimatory decrease in Rubisco activity with growth under elevated CO_2 (Riviere-Rolland *et al.* 1996; Drake *et al.* 1997; Nakano *et al.* 1997; Farage *et al.* 1998; Moore *et al.* 1999; Geiger *et al.* 1999; Pérez *et al.* 2005) will restrict the increase in electron fluxes for CO_2 and O_2 fixation and, if the decrease in Rubisco is strong, will decrease electron transport relative to plants under ambient CO_2 conditions (Hymus *et al.* 2001).

Increases, or at least no decrease, in photochemical activity in plants grown and measured under elevated CO_2 conditions have been reported frequently (Habash *et al.* 1995; Faria *et al.* 1996; Gouk *et al.* 1999; Luomala *et al.* 2003; Usuda 2004; Centritto 2005; Zhang and Dang 2006). However, photosynthetic acclimation to elevated CO_2 has been shown to lead to a decrease in the electron transport rate (Roden and Ball 1996; Scarascia-Mugnozza *et al.* 1996; Kitao *et al.* 2005; Aranda *et al.* 2006; Bigras and Bertrand 2006). This has been reported by some authors using measurements at a common CO_2 concentration in plants grown in elevated and ambient CO_2 conditions (Spunda *et al.* 1998; Aranda *et al.* 2006), allowing a distinction to be made between the direct (short term) and acclimatory (long-term) effects of atmospheric CO_2 enrichment. In some (Bartak *et al.* 1996; Hogan *et al.* 1997; Usuda 2004; Kitao *et al.* 2005; Martinez-Carrasco *et al.* 2005), but not all (Spunda *et al.* 1998; Bigras and Bertrand

2006), the preceding reports, maximal photochemical efficiency in the dark adapted state (Fv:Fm) did not change with down-regulation of photosynthesis under elevated CO_2, suggesting that variations in electron transport would be due to the capacity to use its products rather than to changes in the photosynthetic apparatus. Modifications in the quantum yield of Photosystem II (PSII) electron transport (Φ_{PSII}) induced by growth under elevated CO_2 have been related to changes in photochemical quenching (qP) rather than in maximum photochemical efficiency in light (Fv':Fm') (Hymus *et al.* 1999; Kitao *et al.* 2005; Aranda *et al.* 2006). In contrast, we have previously found (Martinez-Carrasco *et al.* 2005) that Fv':Fm', measured under ambient CO_2 in wheat plants grown under elevated CO_2, increased at ear emergence and decreased soon after anthesis, together with decreases in qP at ear emergence. After anthesis, qP did not decrease in one of the years studied, while it was lower under elevated than under ambient CO_2 late in grain growth in another year. The question remains as to whether decreases in qP may also occur soon after anthesis. Increases in Fv':Fm', accompanying decreases in qP, may involve an increased transfer of excitation energy from the pigment bed to a smaller fraction of open PSII centres, as has been reported for water stress (Giardi *et al.* 1996; Sánchez-Rodriguez *et al.* 1997). They would also suggest that growth under elevated CO_2 changes the relative size of the PSII light-harvesting complex. Habash *et al.* (1995) reported no effect of elevated CO_2 on Fv':Fm' or the chlorophyll a:b ratio in the second fully expanded leaves of wheat six weeks after sowing. In contrast, Bigras and Bertrand (2006) found a decrease in this ratio – consistent with a relative increase in the light-harvesting antenna - in association with a down-regulation of photosynthesis under elevated CO_2 in *Picea mariana*. Likewise, we have found (Pérez *et al.* 2007) a lower chlorophyll a:b ratio, together with increased

Φ_{PSII}, in wheat flag leaves after, but not before, anthesis. Our measurements, however, were conducted only at low light intensity and Fv':Fm' and qP were not determined.

Above-ambient temperatures decrease Φ_{PSII} without affecting Fv:Fm in *Lolium perenne*, attributed to a down-regulation of carbon assimilation caused by the treatment (Bartak *et al.* 1996). Similarly, with mild drought or no water stress, growth at 32 ºC as compared to 26 ºC does not significantly affect Fv/Fm, but significantly decreases Φ_{PSII}, regardless of soil moisture in the perennial grass *Leymus chinensis* (Xu and Zhou 2006). qP was also reported to be decreased by a 4 ºC temperature increase in *Lolium perenne* (Nijs and Impens 1996). In a boreal environment, increasing the temperature by 2 to 6 ºC in field chambers increased Fv:Fm and chlorophyll a+b contents in Scots pine needles (Wang *et al.* 2003). In previous studies with wheat (Pérez *et al.* 2007), chlorophyll fluorescence measurements at low light intensities and ambient CO_2 revealed a negative effect of high temperatures on Φ_{PSII} in plants grown in ambient CO_2, but not under elevated CO_2. Whether this positive interaction of warm temperatures and elevated CO_2 occurs at high irradiance warrants further research. Growth in a CO_2-enriched atmosphere affords protection from the short-term effects of high temperature on net carbon uptake and Fv:Fm (Faria *et al.* 1996; Huxman *et al.* 1998); the beneficial effect of elevated CO_2 was also observed for Φ_{PSII} (Huxman *et al.* 1998). In contrast, Roden and Ball (1996) found a lower Fv:Fm during a heat stress treatment in *Eucalyptus macrorhyncha* grown in elevated as compared with ambient CO_2.

The aim of this work was to know whether high CO_2 and warmer temperatures modify the photochemical efficiency, or only the use of electron transport products, or both. Chlorophyll fluorescence and carbon assimilation were measured in flag leaves at ear emergence and 10-13 days after anthesis, and growth parameters were recorded at ear emergence and maturity. The photosynthetic performance of flag leaves in the

period after ear emergence is of paramount importance for grain yield. We determined chlorophyll fluorescence in the dark-adapted state and in response to a wide range of light intensities. Differing responses at low and high light intensity can help to distinguish between environmental effects on the photochemical efficiency and the use of photochemistry products. In addition, we analyzed chlorophyll contents and chlorophyll a:b ratios, as an index of changes with treatments in the balance between PSII reaction centres and antennae.

Materials and Methods

This study was conducted in two field experiments in different years. The experiment site, located on the farm of the Institute of Natural Resources and Agrobiology, CSIC, in Salamanca (40 º 95 ‘ N, 5 º 5 ‘ W, 800 m a.s.l.), has a clay-sand soil. The climate corresponds to a Mediterranean type. The long-term (20 year) average for the minimum temperature in the coldest month (January) is 0.0 ºC and the maximum temperature of the warmest month (July) is 27.2 ºC. Mean annual rainfall is 506 mm.

Spring wheat (*Triticum aestivum* L cv. Gazul) was sown at a rate of 180 kg ha^{-1} and 0.13 row spacing on 29 January 2004 and 25 January 2006. Before sowing, 60 kg ha^{-1} each of P and K, in both years, and 32 kg ha^{-1} N in 2004 were applied. An application by hand of nitrogen fertilizer [$Ca(NO_3)_2$] as an aqueous solution was performed, at the two different amounts indicated below, on 21 April 2004 and 27 March 2006. Ten days after sowing, herbicides (clortoluron + diflufenican, 2.3 l ha^{-1}) were added; insecticides were applied as required. The crop was watered weekly with a drip irrigation system, providing the amount of water required to equal the average rainfall for each particular month (February, 24.4 mm; March, 25.6 mm; April, 49.7 mm; May, 57.8 mm, and June, 34.3 mm). This represented a low water supply at later growth stages.

After seedling emergence, six temperature-gradient chambers (Aranjuelo *et al.* 2005; Pérez *et al.* 2005), based on those described by Rawson *et al.* (1995), were mounted over the crop at different field sites each year. The chambers were 9m long, 2.2m wide and 1.7m high at the ridge. The chambers had transparent polycarbonate walls and polyethylene sheet roofing, and comprised three consecutive modules (each 3m long) separated by horizontally slotted polycarbonate septa to reduce the mixing of air between modules through convection. Inlet fans and outlet fans and heaters kept the inlet module at temperatures similar to those in the outside air and the final outlet

module at 4 ºC higher temperatures; the central module was left as a spacer. Three chambers were kept at ambient CO_2 (370 μmol mol^{-1}) while in the other three atmospheric CO_2 was increased to 700 μmol mol^{-1} (elevated CO_2) by injecting pure CO_2 at the two inlet fans during the light hours. Since there appear to be no, or only small, positive (Davey et al., 2004) direct effects of growth CO_2 on leaf dark respiration, reports of their presence in the literature seeming to refer to artefacts (Jahnke and Krewitt, 2002), the lack of CO_2 enrichment during the night is probably irrelevant. The CO_2 concentrations and temperatures recorded in the chambers were very close to set values, as in our preceding experiments with these temperature-gradient chambers (Fig. 1 in Del Pozo *et al.* 2005; Fig. 2 in Pérez *et al.* 2005). Two levels of nitrogen supply were established by adding 108 and 140 kg ha^{-1} in 2004 and 2006, respectively, to one longitudinal half of the chambers, and none and 15 kg ha^{-1} in 2004 and 2006, respectively, to the other half, affording total N amounts of 140 and 32 kg ha^{-1} in 2004 and 140 and 15 kg ha^{-1} in 2006. Less fertilizer was used in 2006 than 2004 because of the probability of a carry-.over from 2004 and 2005 to 2006. Control systems were as described (Pérez *et al.* 2005). The CO_2 concentrations and temperatures recorded in the chambers were homogeneous within each chamber module and close to set values (data not shown). The crops achieved densities of 465 ± 13 (2004) and 367 ± 7 (2006) shoots m^{-2}, reaching ear emergence and anthesis four days earlier than the plants outside the chambers.

Chlorophyll fluorescence measurements

At ear emergence (24-28 May 2004 and 15-17 May 2006) and 10 to 13 days after anthesis (7-10 June 2004 and 29 May to 1 June 2006), chlorophyll fluorescence was measured with a modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) in the

central segment of two flag leaves (subsamples) of each CO_2, temperature and nitrogen combination in the three replicate chambers (48 recordings), between 3 and 8 h after the start of the photoperiod. All the leaves were measured with the same, ambient CO_2 concentration. For this, CO_2 fumigation was temporarily suspended in the chamber where fluorescence was recorded. For measurements in the dark-adapted state, leaf sections were darkened for 20 min with leaf clips. Then, Fo was recorded and a saturating flash of light (several thousands μmol m^{-2} s^{-1}) was applied for 0.8 s to determine Fm. Fo and Fm represent, respectively, the minimal and maximal fluorescence in the dark-adapted state. From these, Fv:Fm [(Fm-Fo)/Fm], the maximal photochemical efficiency, was calculated. Light-adapted leaves were illuminated with the red actinic light source of the fluorometer and protected with a radiation shield to obtain an irradiance of 220 μmol m^{-2} s^{-1}. Saturating light pulses were given every 20 s until steady-state chlorophyll fluorescence parameter values were obtained (about 6 min), recording the fluorescence values immediately before (Fs, steady-state fluorescence) and after (Fm', maximal fluorescence in the light) each pulse. Then, the leaf was covered with a black cloth, the actinic light was switched off, and an infrared light was switched on for 3 s to quickly reoxidize the PSII centres and measure Fo', the minimal fluorescence with a non-photochemical quenching similar to that found in the steady-state under light. The equipment determines Φ_{PSII} [(Fm'-Fs)/Fm'], the quantum yield of PSII electron transport. We calculated photochemical quenching qP [(Fm'-Fs)/(Fm'-Fo')] and Fv':Fm' [(Fm'-Fo')/Fm'], the photochemical efficiency under light. It should be noted that Φ_{PSII} = Fv':Fm' · qP. Non-photochemical quenching (NPQ) equates to (Fm/Fm')-1.

In the second experiment (2006), rapid fluorescence – light responses were recorded. In addition to estimating the maximum rate of electron transport and the quantum yield

under saturating irradiance (Rascher *et al.* 2000), fluorescence-irradiance response curves without prior darkening of leaves allowed the maximum quantum yield, qP and Fv':Fm' to be calculated in light-adapted leaves. Leaves were placed in the clip (2030-B, Walz), were rapidly covered with a black cloth, and illuminated with the PAM-2000 light sources through fibre optics. The red LED was used to obtain irradiances up to about 150 μmol m^{-2} s^{-1}, and the white halogen source for higher light intensities. The change of light source was justified because the LED light does not provide higher light intensities and the halogen light does not allow an adequate range of low-intensity irradiances to be obtained. Although red light might not be sufficient to induce stomatal opening and might thus lead to limiting CO_2 supplies, it is assumed that this was unlikely under the low light-intensity range in which the red LED was used. Light was increased in 11 20 s-steps separated by saturating pulses, from less than 10 to about 2500 μmol m^{-2} s^{-1}. Subsequently, the leaf was darkened to determine Fo' by the procedure described. The parameters Φ_{PSII} and Fv':Fm' were obtained and from these qP was calculated (see above). Chlorophyll fluorescence parameters in dark-adapted leaves were obtained at ear emergence (not after anthesis) following the procedure described for the 2004 experiment.

Gas exchange measurements

Gas exchange of leaves was recorded on the same hours and dates and with the same sampling scheme as chlorophyll fluorescence. Measurements were carried out in the central segment of flag leaves with an air flow rate of 300 ml min^{-1}, 1500 μmol m^{-2} s^{-1} irradiance, and a 1.6 ± 0.23 kPa vapour pressure deficit, using a 1.7 cm^2-window leaf chamber connected to a portable infrared gas analyzer (CIRAS-2, PP Systems, Hitchin, Herts., UK) with differential operation in an open system. Temperature was kept at 25

ºC with the Peltier system of the analyzer. Photosynthesis was recorded at 370 and 700 μmol mol^{-1} CO_2.

Chlorophyll concentration

At mid-morning on the dates of fluorescence and gas exchange measurements in the second year, two subsamples of flag leaves, each consisting of four leaves, were harvested from each treatment combination and replicate, and were rapidly plunged *in situ* into liquid nitrogen and then stored at -80 ºC until analyzed. The fresh weight, leaf area (image analysis), and total chlorophyll, chlorophyll a and chlorophyll b in acetone extracts (Arnon 1949) of frozen subsamples were determined as described (Pérez *et al*. 2005). This allowed the results to be expressed on a leaf-area basis.

Green area and dry matter

At ear emergence and at maturity, samples were harvested to determine the area of green tissues and dry matter accumulation. At ear emergence, the number of shoots in 50 cm of two adjacent rows was counted and five consecutive shoots were harvested from each row. The green area of leaves, stems and ears was measured with an electronic planimeter (LI-3050A, Li-Cor, Lincoln, Nebraska, USA) and the dry weight recorded after drying in an oven at 60 ºC for 48 h. At maturity, all shoots in 50 cm of two adjacent rows were harvested, separated into ears and straw, dried at 60 ºC for 48 ºC and weighed.

Experimental design and statistical analyses

The design of the experiment was a randomized-block strip-plot design with three blocks, the two atmospheric CO_2 concentrations (one chamber each) allocated to whole-

plots within blocks, temperature and nitrogen as rows and columns within whole-plots; the subsamples in subplots within rows and columns, and sampling dates in a stratum within subplots. Variance was estimated using the method of residual maximum likelihood (REML; Genstat 6.2). The effects of factors and factor interactions were tested with the Wald statistic, which corresponds to the factor or interaction sum of squares divided by the stratum mean square, and the respective χ^2 probabilities were obtained.

Fluorescence-light responses in the 2006 experiment were analyzed through regressions (exponential curve of the shape $y=a+br^x$) fitted with Genstat 6.2. The curves fitted to each treatment were compared through an analysis of parallelism (Genstat 6.2) to assess whether common or different parameters (only the *a* regression parameter; both *a* and *b* parameters; or all three parameters) should be fitted to each treatment. The significant regression model with greatest parameter separation should be selected. If only the *a* parameter differs among treatments, then parallel curves are obtained which differ in the maximum value. With more differing parameters for each treatment, the curves also show differences in the slope of the light response. The change from red LED to white halogen light sources caused discontinuities in the response of the fluorescence parameters to irradiance. Accordingly, light responses under (LED) and above (halogen) about 150 μmol m^{-2} s^{-1} were analyzed separately.

Results

Chlorophyll fluorescence

Chlorophyll fluorescence measurements at ambient CO_2 concentration in all treatments allowed the acclimatory changes induced by growth conditions to be estimated. The nitrogen supply had no significant effect on the chlorophyll fluorescence parameters or their response to light intensity in either of the two experiments reported here (data not shown). Therefore, only the CO_2 and temperature effects will be described. In the 2004 experiment, the values for Φ_{PSII}, Fv':Fm' and qP were generally higher, and those for Fv:Fm were lower after anthesis than at ear emergence (Table 1); the NPQ values decreased in ambient CO_2, and increased in elevated CO_2 from ear emergence to anthesis. The reasons for these changes over time are not clear. At ear emergence in 2004, growth in elevated CO_2 significantly decreased Φ_{PSII} (Table 1) as a consequence of decreased qP. In contrast, CO_2 enrichment increased the maximal photochemical efficiency, Fv:Fm. After anthesis, the negative effect of elevated CO_2 on qP was also significant, while Fv':Fm' and NPQ were higher in elevated than ambient CO_2. The contrasting effects of elevated CO_2 on qP and Fv':Fm' resulted in little effect on Φ_{PSII}. CO_2 enrichment increased Fv:Fm to a greater extent after anthesis than at ear emergence. Growth temperature had no significant effects on chlorophyll fluorescence parameters during either of the two growth stages.

To further explore whether CO_2 and temperature modified the efficiency of photochemistry or the demand for the products of electron transport, in a new experiment we measured chlorophyll fluorescence quenching at a range of light intensities, since electron sinks are more likely to limit electron transport at high than low irradiance. In the 2006 experiment, with measurements at ambient CO_2, increasing light intensities decreased the quantum yield of photosynthetic electron transport (Φ_{PSII})

throughout the range of irradiances (Figs. 1 a, b and 2 a, b). Fv':Fm' showed little change with light in the range under 150 μmol m^{-2} s^{-1} (Figs. 1 c and 2 c) and, in contrast, decreased with higher irradiance (Figs. 1 d and 2 d). At ear emergence, the increases in NPQ with light intensity (Fig. 1 h) were much greater than the decreases of Fv':Fm'. The decrease in qP with irradiance (Figs. 1 e, f and 2 e, f) was greater than that of Fv':Fm', such that the decline in Φ_{PSII} with light can mainly be attributed to the reduction in qP.

At ear emergence in the 2006 experiment, elevated CO_2 slightly, but significantly, (Table 2) increased Φ_{PSII} (Fig. 1 a, b). After anthesis, Φ_{PSII} at low light was also higher in elevated than ambient CO_2 (Fig. 2 a). With light intensities above about 150 μmol m^{-2} s^{-1} the difference was reversed, such that elevated CO_2 decreased Φ_{PSII} (Fig. 2b). In both growth stages, the differences in Φ_{PSII} between CO_2 levels were due to the maximum (parameter *a*) for the regression over light intensity ($y = a + br^x$, Table 2) rather than to changes in the rate of decrease with irradiance. Like Φ_{PSII}, Fv':Fm' increased in elevated CO_2 (Figs. 1 c, d and 2 c, d) due to higher initial values (regression parameter *a*, Table 2) and, with low light intensity at ear emergence, to slower decreases with light in elevated than ambient CO_2 (Fig. 1 c; Table 2, different *a* and *b* regression parameters). Treatment differences in Φ_{PSII} were therefore associated with higher Fv':Fm' values in elevated than ambient CO_2 throughout the range of light intensities both at ear emergence and after anthesis (Figs. 1 c, d and 2 c, d). At ear emergence, qP showed a significant difference between ambient and elevated CO_2 in the *a* and *b* regression parameters (Table 2). This implies that with low light intensities (Fig. 1 e) qP was higher for elevated CO_2, but underwent a greater decline with irradiance, in such a way that with higher light intensities (Fig. 1 f) qP was lower in elevated than ambient CO_2. After anthesis, the initial qP values under low light (Fig. 2 e) were no longer

different between ambient- and elevated-CO_2 plants. As at ear emergence, the CO_2 effect with light intensities above 150 μmol m^{-2} s^{-1} (Fig. 2 f) became significant and was reversed, qP being lower with elevated than ambient CO_2; the difference was due to the *a* regression parameter (Table 2) and not to different slopes in the light response. Thus, after anthesis the higher Fv':Fm' in elevated than ambient CO_2 was combined with similar (low light) or lower (moderate or high light) qP in elevated CO_2. This led to an enhancement (low light) or inhibition (high light) of Φ_{PSII} in response to CO_2 enrichment. The increase with CO_2 enrichment in Fv:Fm (data not shown) was small and did not reach statistical significance at ear emergence.The NPQ response to light intensity at ear emergence displayed lower values (smaller *a* regression parameter) with elevated than ambient CO_2 and similar slopes (*b* and *r* parameters) with both CO_2 concentrations (Fig. 1 g, h). After anthesis Fv:Fm and NPQ were not recorded.

At ear emergence in the 2006 experiment, temperatures 4 ºC above ambient values increased Φ_{PSII} (Fig. 1 a, b) through an increase in the *a* regression parameter (Table 2); namely, in its maximal value. After anthesis, the differences between growth temperatures in Φ_{PSII} at low light did not reach statistical significance. With moderate or high light intensities after anthesis, warmer temperatures decreased Φ_{PSII} in elevated CO_2, while this effect did not reach significance in ambient CO_2 (Fig. 2 b). At ear emergence, Fv':Fm' increased with warmer temperatures (Fig. 1 c, d), except at low light intensities in elevated growth CO_2. Among ambient CO_2-grown plants, the initial (below 150 μmol m^{-2} s^{-1} irradiance) decrease in Fv':Fm' with light was lower at warmer temperatures (Fig. 1 c; significant difference in the *a* and *b* parameters of the regression, Table 2). After anthesis, Fv':Fm' was not significantly affected by the growth temperature (Fig. 2 c, d). Above-ambient growth temperatures had no significant effect on qP at ear emergence (Fig. 1 e, f) or, under low light, after anthesis (Fig. 2 e). With

moderate and high light intensities after anthesis, warmer temperatures had a negative effect on qP in plants grown in ambient CO_2 (Fig. 2 f), but no significant effect in elevated CO_2-grown plants. At ear emergence, Fv:Fm was not significantly affected by temperature. The NPQ light response at low irradiances (Fig. 1 g) showed no significant change with the growth temperature. At higher irradiances (Fig. 1 h), warmer temperatures decreased NPQ (lowered the *a* regression parameter, Table 2) both in ambient and elevated CO_2. Fluorescence parameters in the dark-adapted state were not determined after anthesis

Photosynthetic carbon assimilation

In both experimental years, when measured at the respective growth CO_2 concentrations, carbon fixation increased with elevated CO_2 by 62% to 72% (data not shown). To assess the acclimatory effects of growth CO_2, comparisons were made at a common CO_2 concentration of 370 μmol mol^{-1}, at which chlorophyll fluorescence measurements were also performed. Photosynthesis declined between ear emergence and 10-13 days after anthesis (Table 3). Growth in elevated CO_2 decreased carbon fixation measured in ambient CO_2 at ear emergence in 2004 and 2006 and 10-13 days after anthesis in 2004; the effect did not reach significance after anthesis in 2006. This decrease in carbon fixation was not accompanied by decreases in intercellular CO_2 concentrations (C_i, Table 3), pointing to a non-stomatal limitation to photosynthesis in elevated growth CO_2, in agreement with the data for stomatal conductance (g_s, Table 3). In the first year, above-ambient temperatures increased photosynthesis and g_s at ear emergence, but not after anthesis, while increasing C_i on both dates (Table 3). Thus, warmer temperature-enhanced CO_2 diffusion through stomata resulted in faster

photosynthesis rates at ear emergence in 2004. Elevated temperatures had no significant effect on carbon assimilation, C_i or g_s in 2006.

Chlorophyll content and the chlorophyll a:b ratio

From ear emergence to 10-13 days after anthesis in the 2006 experiment, total chlorophyll and chlorophyll a contents per unit leaf area (Table 4) decreased in ambient but not in elevated CO_2; the decrease with time in chlorophyll b was similar in both growth CO_2 concentrations. In turn, the chlorophyll a:b ratio increased after anthesis relative to ear emergence. Total chlorophyll and chlorophyll a and b contents per unit leaf area at ear emergence and after anthesis (Table 4) were higher in elevated than ambient CO_2 in plants grown in ambient temperatures, and they showed no differences due to CO_2 in those grown in warmer temperatures. Elevated CO_2 decreased the chlorophyll a:b ratio. There were no effects of temperature on chlorophyll contents or ratios.

Green area and total dry matter

Growth in elevated CO_2 increased total plant dry matter at ear emergence and maturity without significantly affecting the green area at ear emergence (Fig. 3) in both years. Warmer temperatures had no significant effect on the green area or dry matter.

Discussion

Measurements in ambient CO_2 and moderate or high irradiance have shown that photochemical quenching of fluorescence decreases in plants grown in elevated CO_2, in most cases leading to lower quantum yields of Photosystem II electron transport. Although the magnitude of these changes was only small, in the long term they could have an effect on carbon gain. When compared at the same CO_2 concentration, light-saturated carbon assimilation was also decreased by elevated growth CO_2 while C_i increased (Table 3), indicating that photosynthetic capacity was down-regulated, as observed in many previous studies (Drake *et al.* 1997; Long *et al.* 2004; Del Pozo *et al.* 2005; Pérez *et al.* 2005). The amount and activity of Rubisco also decreased in elevated CO_2 in our earlier experiments under conditions similar to those of the present study (Pérez *et al.* 2005; Alonso et al. 2008) In contrast with the results of Aranda *et al.* (2006), however, elevated CO_2 did not decrease qP at irradiances under about 150 μmol m^{-2} s^{-1}. The capacity to use electron transport products is known to progressively limit photochemistry as light intensity increases (Lawson *et al.* 2002; Baker and Oxbourgh 2004). Thus, the shift from higher, or similar, to lower qP in elevated than ambient CO_2 as irradiance increased implies that restrictions in the carbon assimilation capacity under CO_2 enrichment decreased the use in photochemistry of the light absorbed by the PSII antenna. While qP is generally much more affected by the effective rate constant for photochemistry than by the rate constant for non-radiative dissipation (Baker and Oxbourgh 2004), after anthesis in 2004 qP decreased and NPQ increased in elevated CO_2, suggesting that limitations in carbon assimilation decreased photochemical quenching and that the energy was dissipated non-photochemically (but see below for a discussion of the value of NPQ as an indicator of non-photochemical quenching). In contrast with our results, qP does not decrease in wheat leaves when growth in elevated

CO_2 causes no downward acclimation of carbon assimilation (Habash *et al.* 1995). In spite of photosynthetic acclimation, carbon assimilation (measured at the respective growth CO_2 concentration) and total dry matter (Fig. 4) were higher in elevated than ambient CO_2, as found in other studies (Long et al. 2004). The observed decreases in qP and Φ_{PSII} in elevated CO_2 are therefore likely to disappear in comparisons at the respective growth CO_2 concentrations.

Unlike qP, the maximum photochemical efficiency in light did not decrease, but increased with elevated CO_2 at all light intensities when measured in ambient CO_2. Fv':Fm' can be determined by the rate constants for both photochemistry and non-radiative decay in PSII (Baker and Oxbourgh 2004). NPQ was decreased by elevated CO_2 at ear emergence in 2006. However, NPQ does not allow direct evaluation of the proportion of the change in Φ_{PSII} that can be attributed to changes in non-photochemical quenching, whereas Fv′/Fm′ does (Baker et al. 2007). The fact that Fv':Fm' was also higher in elevated than ambient CO_2 in conditions (high light; decreased Φ_{PSII} after anthesis, Figs 1 and 2) that could enhance non-radiative energy dissipation, strongly suggests that growth in elevated CO_2 increases the photochemical efficiency of PSII. Measurements of Fv:Fm in the 2004 experiment support this conclusion. Reported responses to elevated CO_2 of photochemistry include: a) only small changes in chlorophyll fluorescence, in spite of the lowered Rubisco content in elevated CO_2 (Tezara *et al.* 2002); b) no alterations in Fv':Fm' (Habash *et al.* 1995; Hymus *et al.* (1999); and c) increases in photochemical efficiency, together with an enhanced carboxylation capacity (Zhang and Dang 2006). To our knowledge, the fact that growth in elevated CO_2 decreases the sink for electron transport products, but increases the maximal PSII photochemical efficiency, is a new finding.

It seems likely that the changes in the chlorophyll a:b ratio (Table. 4) are caused by changes in the PSII light-harvesting antenna rather than by changes in the PSII:PSI ratio. As a sensitive indicator of changes in the PSII light-harvesting complex (Habash *et al.* 1995), the decrease in chlorophyll a:b ratios caused by elevated CO_2 pointed to an increase in these complexes with respect to the PSII reaction centres, which could account for the enhancement of photochemical efficiency. Similar increases in light-harvesting complexes in elevated CO_2 have been found in shaded lower leaves of wheat after anthesis (Osborne *et al.* 1998; Adam *et al.* 2000). We have previously found decreased chlorophyll a:b ratios late in the development of wheat leaves in elevated CO_2 (Pérez *et al.* 2007), but Fv':Fm' and qP were not determined. In contrast to our findings, Habash *et al.* (1995) found no changes in chlorophyll a:b ratios caused by elevated CO_2. A decrease in carboxylation capacity, together with an increase in photochemical efficiency and the antenna complexes, suggests a shift towards resource allocation for photon capture in plants grown in elevated CO_2. Although a change in balance between Rubisco and electron transport with atmospheric CO_2 enrichment is not consistent with some reports (Sage *et al.* 1995, Nakano *et al.* 1997), it is in agreement with the results of Makino *et al.* (1997), Osborne *et al.* (1998), and Mitchell *et al.* (2000).

Notably, at ear emergence in 2006 there were greater decreases with elevated CO_2 in carbon fixation than in Φ_{PSII} when measured in ambient CO_2, suggesting a sink other than CO_2 assimilation for electron transport products. Acclimatory decreases in Rubisco in elevated CO_2 should prevent increases in electron flow to the photorespiratory carbon oxidation cycle. Indirect estimates indicate significant water-water cycle (Mehler ascorbate peroxidase) activity when CO_2 assimilation is restricted (Ort and Baker 2002), but no effect of elevated CO_2 on alternative electron sinks has been found previously (Habash *et al.* 1995; Hymus *et al.* 1999). Further combined measurements of

chlorophyll fluorescence, carbon assimilation and activities of the enzymes involved in the Mehler pathway are required to gain (indirect) evidence of changes in alternative electron sinks in leaves acclimated to elevated CO_2.

In the 2006, though not in the 2004 experiment, warmer temperatures elicited an effect on chlorophyll fluorescence that was dependent on leaf age and, at later growth stages, on CO_2. At ear emergence, the increase in Φ_{PSII} with temperature in measurements with ambient CO_2 was due to an increase in Fv':Fm' at all light intensities, with no change in qP, suggesting that the use of electron transport products was not decreased by temperature. In agreement with this, carbon assimilation was not affected, or tended to increase, in ambient + 4 ºC temperatures. The decrease with temperature in NPQ (Fig 1) was consistent with a decrease in non-photochemical quenching. After anthesis, the positive effect of temperature on Fv':Fm' disappeared, tending to be reversed in plants under elevated CO_2, in which qP did not change and Φ_{PSII} decreased with temperature. This suggests an increase in non-radiative energy dissipation, which could protect PSII centres from over-reduction. In ambient CO_2 plants, the absence of effects of high temperatures on Fv':Fm' indicated that non-radiative energy dissipation did not change, and this was associated with an increased reduction of PSII reaction centres, as evidenced by the decrease in qP. In previous studies with low measurement light (Pérez et al. 2007), after anthesis we found a negative effect of high temperatures on Φ_{PSII} in ambient CO_2, but not in elevated CO_2, consistent with similar trends in the present experiments (Fig. 2 a). Under high light, by increasing the non-radiative energy dissipation in warmer temperature, elevated CO_2 can protect PSII from damage, as reported by Taub *et al.* (2000). Although above-ambient temperatures did not significantly affect dry matter production, a trend towards decreased growth at high temperatures was observed in both years. Warmer

temperatures are known to enhance growth but cause its earlier cessation (Ford and Thorne 1975). The loss over time in the positive effects of temperature on Φ_{PSII} is consistent with this growth response to warming.

We conclude that future increases in atmospheric CO_2 and temperature may have a positive effect on photochemical efficiency, in spite of the down-regulation of carbon assimilation in elevated CO_2. High CO_2 can mitigate the adverse effect of high temperatures on photochemistry observed in ambient CO_2 late in leaf growth. This work provides evidence that with air CO_2 enrichment a reallocation of resources favouring light capture may occur.

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Figure captions

Fig. 1. 2006 experiment. Responses to low (a, c, e, g) and high (b, d, f, h) light intensities of chlorophyll fluorescence parameters measured in ambient CO_2 in flag leaves of wheat at ear emergence. Wheat was grown in ambient (open symbols) or elevated (closed symbols) CO_2 combined with ambient (circles) or ambient + 4 ºC (squares) temperatures in field chambers. Common or separate regressions were fitted to the treatments according to the analysis of parallelism in Table 2. Vertical bars represent standard errors. Each point is the mean of 12 replicates.

Fig. 2. 2006 experiment. Responses to low (a, c, e) and high (b, d, f) light intensities of chlorophyll fluorescence parameters measured in ambient CO_2 in flag leaves of wheat 10-13 days after anthesis. Wheat was grown in ambient (open symbols) or elevated (closed symbols) CO_2 combined with ambient (circles) or ambient + 4 ºC (squares) temperatures in field chambers. Common or separate regressions were fitted to the treatments according to the analysis of parallelism in Table 2. Vertical bars represent standard errors. Each point is the mean of 12 replicates.

Fig. 3. Percentage change with growth in elevated CO_2 (CO_2) and ambient + 4 ºC temperatures (T4) of total green area (white bars) and dry matter (grey bars) at ear emergence and total dry matter at maturity (black bars) in wheat grown in field chambers in 2004 and 2006. Asterisks represent significant effects.

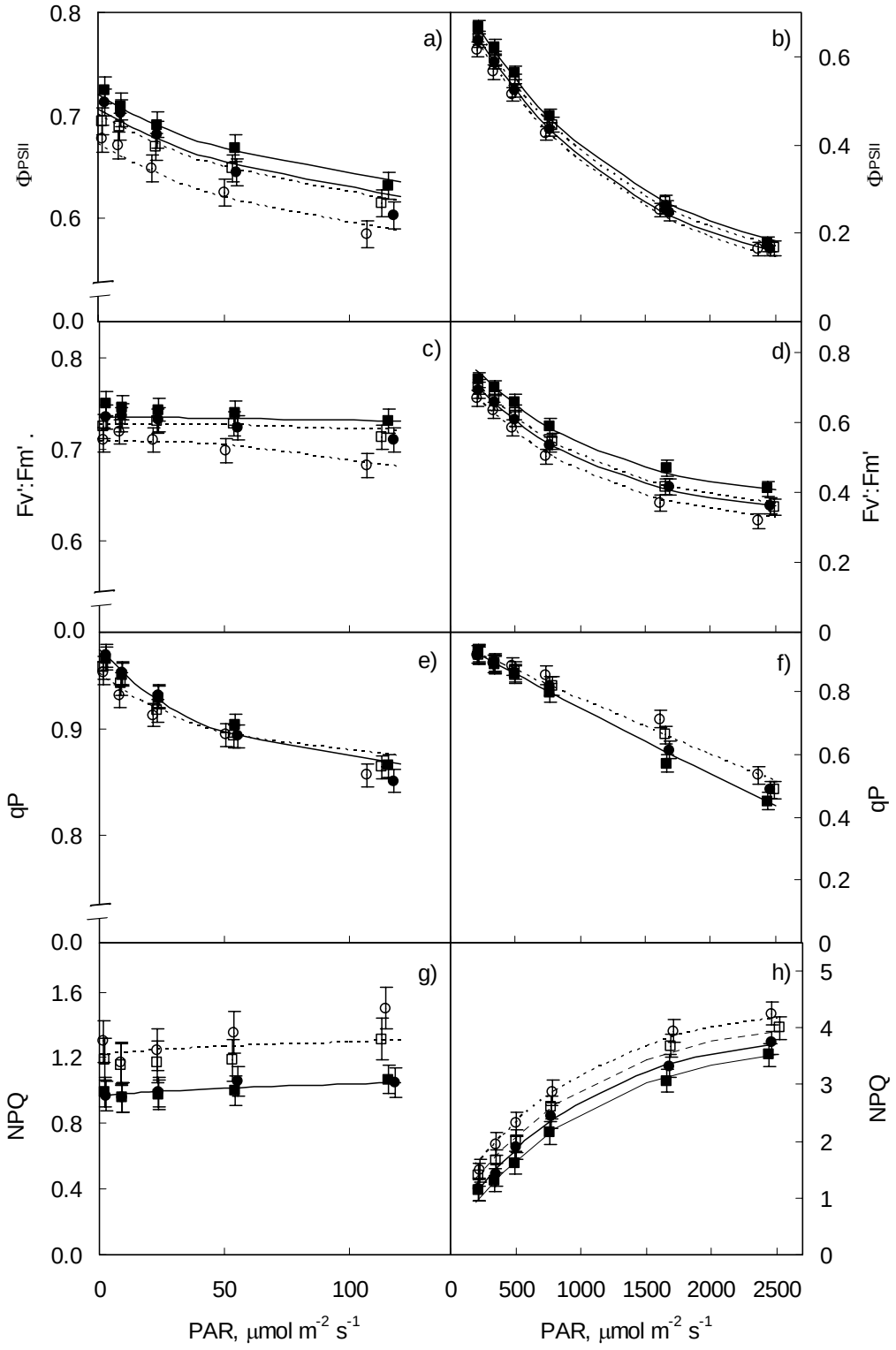

Fig.1

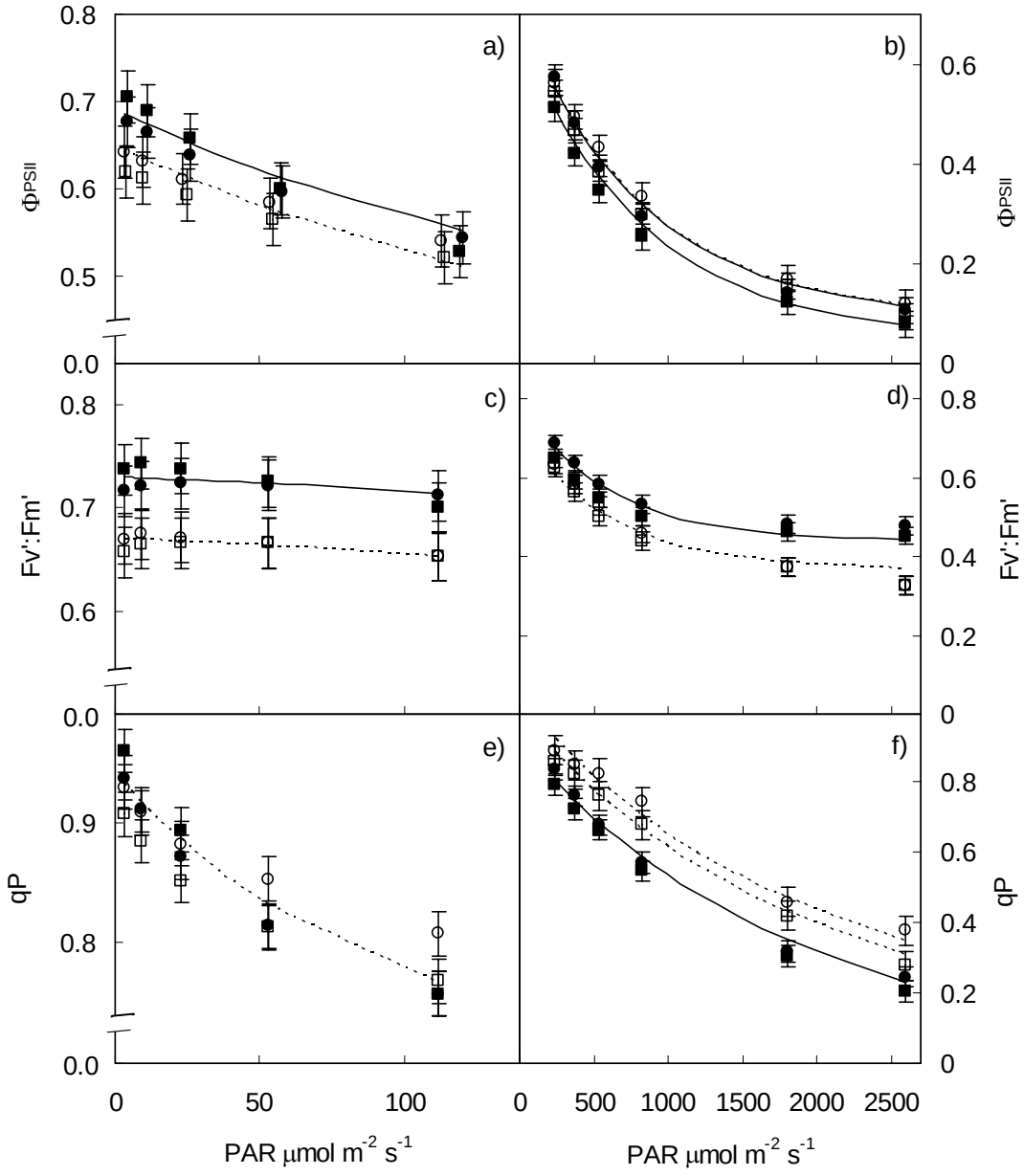

Fig. 2

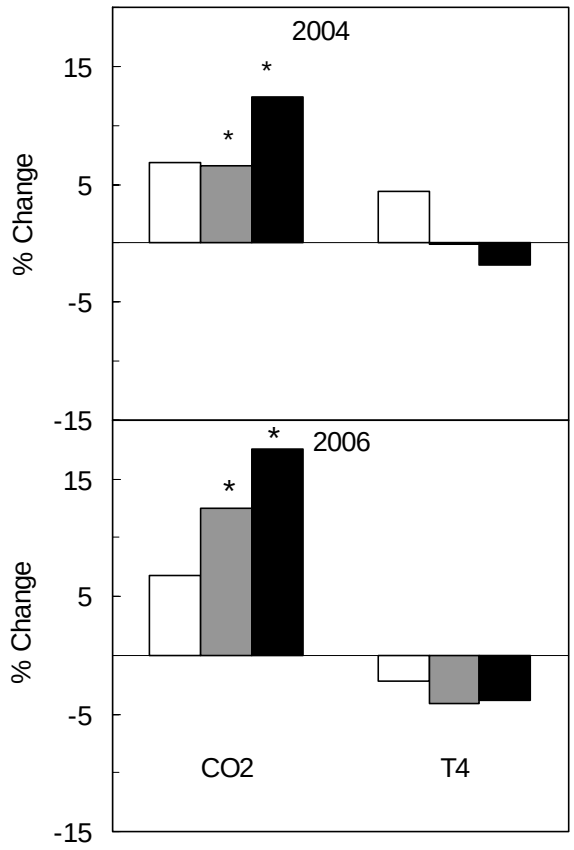

Fig. 3